APPENDIX G. DEVELOPMENT OF CHEMICAL-SPECIFIC METABOLIC BIOTRANSFORMATION RATE CONSTANT ASSUMPTIONS

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1 Introduction

This appendix discusses the development of metabolic biotransformation rate constant (K_M) distributions for use in the bioaccumulation model and describes the available information regarding K_M values for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and tetrachlorobiphenyl (tetraCB) for both fish and invertebrates (including both blue crab and small benthic invertebrates).

2 Metabolic Biotransformation Rate Constants for Fish

Fish K_M values for 2,3,7,8- TCDD and tetraCB for use in the bioaccumulation model were developed based using three main sources:

- **Precedent** – The use of K_M values for modeled chemicals in previous bioaccumulation models were considered.
- **Review of empirical tissue data from the Lower Passaic River Study Area (LPRSA)** – Empirical tissue data were evaluated to determine whether different species uptake and/or metabolize chemicals differently.
- **Available information in literature** – Available information in literature was reviewed to evaluate whether chemicals are metabolized by various species and to select K_M values for use in the LPRSA bioaccumulation model. Arnot et al. (2008b) compiled a database of fish biotransformation rates, which was used as the primary source for assigning K_M values for the LPRSA. This paper presented a comprehensive review of available laboratory data on the metabolic biotransformation of non-ionic organic chemicals by fish and also provided and applied methodology for estimating metabolic rate constants from the data.

Table 1 provides a summary of the selected metabolic rate distributions and the rationale for the development of these distributions. More details are provided in the subsections that follow.

Table 1. Summary of fish K_M distributions

Chemical	Species	K_M Distribution		Summary of Rationale
		Nominal Value	Acceptable Range	
2,3,7,8-TCDD	carp	0.014	0.0016 – 0.056	Species-specific data on metabolic biotransformation rates are available for carp and provide evidence that the K_M values for carp are lower than for other fish (Arnot et al. 2008a; Arnot et al. 2008b), so carp metabolic biotransformation rates were calibrated separately from those of other fish using carp-specific values.
				Available literature and the LPRSA empirical data indicated that the bioaccumulation pattern of eel is different than those of other fish. In a study of European eel (a similar species), Van der Oost et al. (1996) concluded that the low bioaccumulation of dioxins/furans was most likely due to reduced uptake, effective metabolic clearance, or both. No eel-

	American eel		0.0016 – 0.082	specific K_M values were available; thus, high-end estimates (i.e., the 97.5th percentile estimates of K_M) of metabolic biotransformation rates were derived using all fish data from Arnot et al. (2008a). The K_M could represent a truly higher metabolic biotransformation rate, or it could serve as a surrogate for describing another process that results in reduced uptake relative to other fish.
	other fish	0.013	0.007 – 0.024	K_M values were developed using all available K_M values for 2,3,7,8-TCDD (i.e., rates for all available species) from Arnot et al. (2008a).
TetraCB	American eel	0.005	0.0004 – 0.01	Available literature and the LPRSA empirical data indicated that unlike other fish, eel are able to metabolize lower-chlorinated PCBs (such as tetraCBs). No eel-specific rates were available; thus, K_M values for tetraCB congeners from Arnot et al. (2008b) were used to develop the distribution.
	other fish	0	na (point estimate)	Based on a review of the available literature and on precedent from past model applications, metabolism was assumed to be equal to 0.

K_M – metabolic biotransformation rate constant
LPRSA – Lower Passaic River Study Area
na – not applicable
PCB – polychlorinated biphenyl
TCDD – tetrachlorodibenzo-*p*-dioxin
tetraCB – tetrachlorobiphenyl

2.1 2,3,7,8-TCDD

If species-specific K_M values were provided in Arnot et al. (2008b), they were applied to the appropriate species in the bioaccumulation model. This was the case for carp and 2,3,7,8-TCDD, for which species-specific estimated rates were available from three studies (Arnot et al. 2008b). For carp, the nominal value of the distribution was set equal to the average of the best estimate for the three carp-specific studies (Table 2). The range of the distribution was set equal to the range of the estimated 2.5th to 97.5th percentile values. Species-specific rate estimates were not available for any other modeled fish species. For all other fish (with the exception of eel, as discussed below), the nominal value of the distribution was set equal to the average of the best estimates for all species, and the range was set equal to the minimum and maximum best estimates for all fish species reported in Arnot et al. (2008b) (Table 2).

Table 2. Fish K_M values for 2,3,7,8-TCDD from Arnot et al. (2008b)

Species	Temperature and Body-Weight Normalized K_M Values				Data Category[a]
	Best Estimate		Estimated Percentiles		
	log K_M	K_M	2.5th Percentile	97.5th Percentile	
Common carp	-1.72	0.019	0.0063	0.057	1
	-1.85	0.014	0.0044	0.048	1
	-2.12	0.008	0.0016	0.035	1
Fathead minnow	-2.05	0.009	0.0030	0.027	1
	-2.14	0.007	0.0022	0.024	1
Guppy	-2.08	0.008	0.0016	0.044	1

Rainbow trout	-1.62	0.024	0.0071	0.082	1

[a] A data category ranging from 1 (indicating a very high level of confidence) to 5 (indicating a low level of confidence) or 6 (indicating an uncertain level of confidence) was assigned to each study (Arnot et al. 2008b).

K_M – metabolism biotransformation rate constant

TCDD – tetrachlorodibenzo-*p*-dioxin

As noted above, a different K_M distribution was used for American eel and 2,3,7,8-TCDD. Although no species-specific K_M information was available for eel in Arnot et al. (2008b) (Table 2), LPRSA empirical tissue data and other literature information (Van der Oost et al. 1996) supported the use of a different K_M value for eel relative to those for other species.

For each species evaluated in the bioaccumulation model, the ratio of the average empirical tissue concentration to the sediment concentration in the applicable modeling area was calculated. These ratios were compared to evaluate whether the bioaccumulation potential and/or metabolic biotransformation may be different for the various species. The results of this comparison are presented in Table 3, which is ordered based on the ratio of tissue to sediment concentrations (highest for carp and lowest for American eel). Some of the differences in these ratios can be explained by the diets of these fish. For example, carp diets are closely tied to sediment (i.e., carp feed by foraging in the sediment for food, and thus their diet is composed primarily of sediment, near-bottom particulates, and benthic invertebrates). On the other hand, bass diets are less closely linked to sediment (and more closely linked to water column exposures) because their diet is composed of a higher fraction of small fish and higher-trophic-level benthic invertebrates. Other differences, such as the low ratio for American eel, may indicate that bioaccumulation potential and/or metabolic biotransformation is different among species.

Table 3. Ratios of fish tissue to sediment concentrations for 2,3,7,8-TCDD in the LPRSA

Species Group	Average Tissue Concentration (ng/kg ww)	Modeling Area	Sediment SWAC (ng/kg dw)	Ratio of Tissue to Sediment Concentrations
Carp	430	RM 7 – Dundee Dam	1,468	0.29
White perch	130	site-wide	1,000	0.13
Catfish	130	RM 4 – Dundee Dam	1,505	0.09
Bass	30	RM 7 – Dundee Dam	1,468	0.04
American eel	18	site-wide	1,000	0.02

dw – dry weight

LPRSA – Lower Passaic River Study Area

RM – river mile

SWAC – spatially weighted average concentration

ww – wet weight

In a study of the bioaccumulation patterns of various organic compounds in European eel (a species closely related to American eel) (Van der Oost et al. 1996), the

bioaccumulation of dioxins/furans was found to be extremely low. Van der Oost et al. (1996) concluded that this result was most likely due to reduced uptake, effective metabolic biotransformation, or both. Although this study was not sufficient to develop an eel-specific K_M value, it supports the use of a different (i.e., higher) K_M value for eel.

Thus, based on LPRSA empirical tissue data and the available literature information, a distribution that reflected the higher metabolic biotransformation (or lower uptake) potential for American eel was developed. The nominal value for the American eel distribution was set equal to the average of the 97.5th percentile estimates for the available K_M values from Arnot et al. (2008a), and the distribution range reflects the 2.5th and 97.5th percentiles reported for any species (Arnot et al. 2008a) (Table 2).

2.2 TETRACB

In numerous previous applications of bioaccumulation models, the metabolic biotransformation of polychlorinated biphenyls (PCBs) by fish has been assumed to be equal to zero (Arnot and Gobas 2004; Gobas and Arnot 2010; Windward 2010; HydroQual 2007). This precedent was used as the starting point for determining K_M values for tetraCB.

As was done for 2,3,7,8-TCDD, the ratio of the average empirical tissue concentration to the sediment concentration in the applicable modeling area was calculated, and was used to evaluate whether the bioaccumulation potential and/or metabolic biotransformation may be different for the various fish species (Table 4). The resulting pattern is similar to that for 2,3,7,8-TCDD in that the ratio is highest for carp and lowest for bass and American eel. Taking into account feeding strategies and diets, this comparison provides evidence that the bioaccumulation potential and/or metabolic biotransformation for American eel may be different those for other species.

Table 4. Ratios of fish tissue to sediment concentrations for tetraCB in the LPRSA

Species Group	Average Tissue Concentration (µg/kg ww)	Modeling Area	Sediment SWAC (µg/kg dw)	Ratio of Tissue to Sediment Concentrations
Carp	1,090	RM 7 – Dundee Dam	549	1.99
White perch	470	site-wide	518	0.91
Catfish	371	RM 4 – Dundee Dam	624	0.60
Bass	283	RM 7 – Dundee Dam	549	0.52
American eel	184	site-wide	518	0.36

dw – dry weight
LPRSA – Lower Passaic River Study Area
RM – river mile
SWAC – spatially weighted average concentration
ww – wet weight

In addition to the evaluation of LPRSA tissue data, available information from literature was reviewed to evaluate whether eel metabolize PCBs differently as compared with

other fish. Relatively little relevant information was available on this topic.

A review conducted by Brown (1992) examined the activity of the enzyme cytochrome P-4502B (P4502B), which is likely important in the metabolic biotransformation of certain PCBs (including several tetraCBs) by reviewing capillary gas chromatograms (GCs) from previous investigations. Brown (1992) looked at the activity of this enzyme (and attempted to quantify the magnitude of activity when present) in 54 aquatic species, including molluscs, annelids, arthropods, echinoderms, and fish. Of the 32 species of fish evaluated, only 4 (i.e., American eel, cunner, red-breasted sunfish, and winter flounder) showed P4502B activity. The fish species included in this evaluation are presented in Table 5; species modeled as part of the LPRSA bioaccumulation model are in bold.

Table 5. Summary of P4502B activity in fish from Brown (1992)

Species Evaluated in Brown (1992)			
Species for which No P4502B Activity was Observed			**Species for which P4502B Activity was Observed**
American shad	Goldfish	Sheepshead minnow	**American eel**
Arctic cod	Lake trout	**Smallmouth bass**	Cunner
Atlantic silversides	**Largemouth bass**	Striped bass	Red-breasted sunfish
Atlantic tomcod	Lizardfish	Striped killifish	Winter flounder
Bloater	**Menhaden**	Tautog	
Bluefish	**Mummichog**	Walleye	
Brown bullhead	Naked goby	**White catfish**	
Carp	Northern pike	**White perch**	
Crevalle jack	Pumpkinseed	Yellow perch	
Fathead minnow			

Bold identifies species that were modeled as part of the LPRSA bioaccumulation model.
P4502B – cytochrome P-4502B

A literature search was performed to look for additional, more recent investigations of P4502B activity in fish. This search returned limited results. However, one study (Koenig et al. 2012), which evaluated the role of P4502B-mediated metabolic biotransformation in several species of crustaceans and non-eel species of fish, was found. The species of fish evaluated were reported to have a lack of P4502B-like enzymes, even though, in contrast, crustaceans exhibited activity for these types of enzymes. Thus, Koenig et al. (2012) concluded that the differences in PCB profiles between the fish and crustaceans could be linked to variations in P4502B-mediated metabolic biotransformation.

As discussed above, the available literature information and LPRSA data suggest that unlike most fish species, American eel express P4502B, which is associated with the metabolic biotransformation of some PCBs, including several tetraCBs. Thus, a distribution for eel was developed using the available K_M values for tetraCB congeners from Arnot et al. (2008b), which are summarized in Table 6. As indicated in Table 6, nearly all of the available K_M values were based on studies of rainbow trout; rates were not available for any of the fish evaluated in the LPRSA bioaccumulation model. To take into account the prevelance of the various tetraCB congeners in the LPRSA, the

percent contribution to tetraCB sum in sediment was calculated for each each congener (these were then normalized for the congeners for which K_M values were available). A weighted average K_M value of 0.005 was then calculated for tetraCB. Table 6 presents additional summary statistics and the selected distribution values for American eel. The metabolic biotransformation of tetraCB for other fish (i.e., non-eel fish) was assumed to be zero.

Table 6. Fish K_M values for tetraCB congeners from Arnot et al. (2008b)

Congener	No. of Studies	Species	Average K_M	Percent Contribution to TetraCB in LPRSA Sediment	Summary Statistics and Selected Distribution
PCB 40	5	rainbow trout	0.008	8.2%	**Summary statistics** • **Average K_M (all data):** 0.006 • **Average K_M (across congener averages):** 0.005 • **Range:** 0.0003 to 0.03 • **Weighted average K_M:** 0.005 **Selected distribution** • **Best estimate:** 0.005 (based on the weighted average) • **Acceptable Range:** 0.0004 – 0.01 (based on the 10th to 90th percentiles of data for all congeners)
PCB 42	4	rainbow trout	0.002	4.8%	
PCB 44	5	rainbow trout	0.007	21%	
PCB 46	3	rainbow trout	0.005	1.1%	
PCB 52	5	guppy (4), rainbow trout (1)	0.002	19%	
PCB 53	1	rainbow trout	0.0008	0%	
PCB 56	2	rainbow trout	0.003	6.3%	
PCB 61	1	rainbow trout	0.0009	24%	
PCB 65	1	rainbow trout	0.0004	0%	
PCB 66	4	rainbow trout	0.006	14%	
PCB 70	3	rainbow trout	0.003	0%	
PCB 72	1	rainbow trout	0.0003	0.2%	
PCB 74	3	rainbow trout	0.004	0%	
PCB 77	2	rainbow trout	0.03	2.3%	

K_M – metabolism biotransformation rate constant
LPRSA – Lower Passaic River Study Area
PCB – polychlorinated biphenyl
tetraCB – tetrachlorobiphenyl

3 Metabolic Biotransformation Rate Constants for Invertebrates

This section presents the selected K_M values for invertebrates and the process used to develop these values. The K_M values for invertebrates (including both small benthic invertebrates and blue crabs) were developed based on two main sources:

- **Precedent** – K_M values used in previous bioaccumulation models were considered.
- **Literature review** – A review of the available literature was conducted for both dioxins and PCBs for invertebrates (details of these searches are provided later in this section).

A summary of the selected rates and rationales is presented in Table 7. Additional

details for 2,3,7,8-TCDD and tetraCB are provided in the subsections that follow.

Table 7. Summary of invertebrate K_M distributions

Chemical	Species	K_M Distribution		Summary of Rationale
		Nominal Value	Acceptable Range	
2,3,7,8-TCDD	small benthic invertebrates	0.013	0.007 – 0.024	The available literature indicated that invertebrates (including both benthic invertebrates and blue crab) may be able to metabolize dioxins/furans. No invertebrate-specific rates were available, and thus the distribution for fish was also applied to invertebrates (see Table 1 and Section 2.1).
	blue crab			
TetraCB	small benthic invertebrates	0	na (point estimate)	The available literature and precedent based on previous model applications indicated minimal metabolic biotransformation of PCBs by small invertebrates.
	blue crab	0.005	0.0004 – 0.01	The available literature indicated that lower-chlorinated PCBs (including some tetraCB congeners) are likely to be metabolized to some extent by crab. No crab-specific rates were available, and thus the distribution developed for American eel was also applied to crab (see Table 1 and Section 2.2).

K_M – metabolism biotransformation rate constant
na – not applicable
TCDD – tetrachlorodibenzo-*p*-dioxin
tetraCB – tetrachlorobiphenyl

3.1 2,3,7,8-TCDD

Support for the metabolic biotransformation (or inefficient uptake) of dioxins/furans by invertebrates can be found in work performed for the Contaminant Assessment and Reduction Project (CARP) for the New York/New Jersey Harbor estuary (HydroQual 2007). In that study, biota-sediment accumulation factors (BSAFs) for PCB homologues and dioxin/furan congeners for blue crab, clams, and worms were calculated using field-collected tissue data and model-calculated sediment concentrations. The resulting BSAFs were plotted against K_{OW} for the two chemical groups (i.e., PCBs and dioxins/furans). The calculated BSAFs for dioxin/furan congeners for clams, crabs, and worms were approximately 10 times lower than those for PCBs (for chemicals with similar K_{OW}s). The HydroQual (2007) report stated that "this suggests that either there is an inefficient transfer of dioxin/furan congeners from sediment, or that worms also possess the capacity to metabolize dioxin and furan congeners." A similar summary was provided in the same report for clam and crab. It should be noted that the HydroQual (2007) report did not include metabolic biotransformation by zooplankton based on a similar comparison of empirical tissue concentrations and modeled dissolved water concentrations for PCBs and dioxins/furans. This is consistent with the assumption that the K_M for zooplankton is equal to 0 in the LPRSA bioaccumulation model.

As part of the effort to develop K_M values for 2,3,7,8-TCDD, in September 2014, a literature search was conducted for studies on the metabolic biotransformation of dioxins and furans by aquatic invertebrates using the Web of Science database.

Search terms used in this search included the following: dioxin, furan, metabolism, metabolites, metabolic transformation, biotransformation, crayfish, crab, aquatic organism, biota, and bioaccumulation.

CYP450 1A expression (CYP450 1A1 is the most important enzyme in TCDD metabolism for vertebrates) is not known to occur in benthic invertebrates. It is possible that benthic invertebrates metabolize 2,3,7,8-TCDD by a different route than vertebrates. One study (Zhang et al. 2011), which measured the uptake and elimination of a dioxin compound for invertebrates, was found. In this study, radiotracers were used to measure the uptake, assimilation efficiency, and elimination of 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin[1] in marine phytoplankton, copepods, and fish. The half-life of 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin of 2 to 25 days for copepods was lower than that observed for fish in other studies (Zhang et al. 2011). According to Zhang et al. (2011), the results suggested that these invertebrates have a rapid metabolic biotransformation rate due to their small size and may indicate that copepods have an efficient elimination system for removing or metabolizing 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin.

Based on the supporting information summarized above, non-zero K_M values were applied for 2,3,7,8-TCDD for both benthic invertebrates and blue crab. No invertebrate-specific rates could be identified; thus the K_M distributions developed for fish (based on Arnot et al. 2008b) were also used for benthic invertebrates and blue crab.

3.2 TETRACB

As part of developing K_M values for tetraCB, in January 2015, a literature search was conducted for studies on the metabolic biotransformation of tetraCB by aquatic invertebrates using the Web of Science and Google Scholar databases. Search terms used in this search included the following: tetrachlorobiphenyl, PCBs, PCB homologues, Aroclors, metabolism, metabolites, metabolic transformation, biotransformation, crayfish, crab, invertebrate, and bioaccumulation.

This search yielded relatively few articles, most of which focused on the metabolic biotransformation of PCBs by crabs or other decapods. Key points associated with invertebrates and the metabolic biotransformation of tetraCBs are as follows:

- Species-specific studies (Porte and Albaiges 1993; Bodin et al. 2007) indicated that several crab species (i.e., *Macropipus tuberculatus*, *Necora puber*, and *Cancer pagurus*) have a strong capacity to metabolize certain PCB congeners relative to that of mussels and fish.
- TetraCB includes several congeners found to be strongly metabolized in crab (Porte and Albaiges 1993). The position of chlorines on the PCBs is an apparent determinant of metabolic biotransformation (Finley et al. 1997; Porte and Albaiges 1993), with the enzyme cytochrome P-4502B (P4502B) likely being important in that process (Brown 1992; Bodin et al. 2008; Bodin et al.

[1] Zhang et al. (2011) did not identify the specific dioxin compound that was evaluated in this study. In a personal communication, the authors (Wang 2014) clarified that the compound used in their study was to be 1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin.

2007).

- Brown (1992) reported that P4502B activity was not observed in any of the mollusc, annelid, or echinoderm species evaluated but was observed in some larger crustaceans (particularly lobster and blue crab).[2]

Overall, the literature indicated that lower-chlorinated PCBs are likely metabolized to some extent in crab but that metabolic biotransformation is minimal in most other invertebrates. Thus, the K_M values for tetraCB for the small benthic invertebrates included in the bioaccumulation model was assumed to be equal to zero. This was consistent with the assumptions for PCBs used in previous applications of bioaccumulation models (Arnot and Gobas 2004; Gobas and Arnot 2010; Windward 2010; HydroQual 2007). As indicated above, metabolic biotransformation was included for blue crab, based on literature that supported the metabolic biotransformation of tetraCB by crabs. No crab-specific rates could be identified; thus the K_M distribution developed for American eel (based on the available fish data from Arnot et al. 2008b) were also used for blue crab.

4 Related Processes that Influence Chemical Concentrations

When reviewing K_M data, it is important to recognize that other processes that influence chemical concentrations in biota are likely to affect these data. In a study of European eel (discussed in Section 2.1), Van der Oost et al. (1996) noted that the lower chemical concentrations observed in eel could be the result of reduced uptake, high rates of metabolic biotransformation, or a combination of these processes. For the purpose of the bioaccumulation model, it is not necessarily important to distinguish between the metabolic biotransformation rate constant and factors that could reduce the uptake of a given chemical because both processes have the same outcome: a lower concentration of the chemical (i.e., parent/unmetabolized chemical) in biota tissue. However, it is important to acknowledge the overlapping nature of these processes, particularly for parameters such as the metabolic biotransformation rate constant, for which species-specific and/or site-specific data are often unavailable.

Rather than attempting to capture all of the processes that exist in a system (a task that would be nearly impossible), the goal of the bioaccumulation model is to replicate the LPRSA system to the extent necessary to accurately predict tissue concentrations. It is important to add sufficient complexity to ensure that the model can replicate the complex natural system and at the same time not create an unnecessarily complex model. Thus, in cases where chemical-specific K_M values and other factors result in a reduced uptake of chemicals, it is appropriate to use a single parameter to act as a surrogate for related processes.

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[2] Koeing et al. (2012) reported lower levels of P4502B-inducing PCB congeners in one species of shrimp. This was consistent with Brown (1992), which also identified P4502B activity in shrimp.

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